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Contribution of liver nerves, glucagon, and adrenaline to the glycaemic response to exercise in rats

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The contribution of hepatic sympathetic innervation, glucagon and adrenaline to the glycaemic response to exercise was investigated in rats. Hepatically denervated (LDX) or sham operated (SHAM) rats with permanent catheters were therefore submitted to swimming with or without infusion of somatostatin in combination with adrenomedullation. Blood samples were taken for measurements of blood glucose, plasma free fatty acids (FFA), adrenaline (A), noradrenaline (NA), insulin and glucagon. Liver denervation by itself did not influence glucose levels during exercise. Infusion of somatostatin in SHAM animals, which inhibited the exercise-induced glucagon response, led to enhanced sympathoadrenal outflow (measured as plasma A and NA) and a reduced blood glucose during exercise, suggesting that glucagon serves as a powerful mediator of the glycaemic response during swimming. Infusion of somatostatin in LDX animals failed to enhance plasma NA levels and led to a more pronounced reduction in blood glucose levels. This indicates that liver nerves do contribute to the glycaemic response to exercise when glucagon secretion is suppressed. Reduced blood glucose levels after adrenomedullation revealed that adrenal A is another important mediator of the glucose response to exercise. Infusion of somatostatin in adreno-medullated SHAM or LDX animals was not accompanied with increased NA outflow, suggesting that adrenal A is necessary to allow the compensatory increased outflow of NA from sympathetic nerves. In conclusion, the study shows that pancreatic glucagon and adrenal A are the predominant factors influencing the glycaemic response to exercise, whereas a role of the sympathetic liver nerves becomes evident when glucagon secretion is suppressed.

Key words: adrenaline, adrenomedullation, glucagon, glucose, insulin, liver nerves, noradrenaline, somatostatin, swimming, sympathetic nervous system.

Carbohydrates are the predominant fuel for contracting muscles early in exercise. Intracellularly, they derive mainly from breakdown of glycogen in working muscles, whereas circulating glucose stems from glycogenolysis and gluconeogenesis in the liver. As exercise continues, hepatic glucose production becomes more important (Galbo 1983).

Hepatic glucose production is known to be influenced by several factors. First, adrenaline (A) originating from the adrenal medulla is thought to activate hepatic α_1 -adrenoceptors leading to glycogenolysis (Seydoux *et al.* 1979,

305

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Exton 1985). A is also a potent stimulator of glucagon release from the pancreatic islets of Langerhans (Harvey *et al.* 1974, Halter *et al.* 1984), the second factor that stimulates hepatic glucose production (Issekutz & Vranic 1980, Wasserman *et al.* 1989). The importance of glucagon for the glucose response to exercise in rats has been demonstrated by the use of glucagon antibodies (Richter *et al.* 1981). Finally, nor-adrenaline (NA), released from the sympathetic nerves innervating the liver, seems to be the third factor influencing hepatic glucose production (Garceau *et al.* 1983, Shimazu 1983, Winder *et al.* 1983). However, several studies in denervated animals failed to provide direct evidence for a role of hepatic sympathetic nerves in the mobilization of glucose from the liver (Mikines *et al.* 1985, Sonne *et al.* 1985, Wasserman *et al.* 1990). This discrepancy might be explained by compensatory mechanisms that veil the effects of denervation (Seidler & Slotkin 1986, Larsson *et al.* 1989). The present study was designed to investigate the relative importance of hepatic sympathetic innervation and possible compensatory mechanisms in the glycaemic response to exercise. Therefore, concentrations of blood glucose, plasma FFA, A, NA, insulin and glucagon were monitored in hepatically denervated, adrenomedullated and somatostatin infused swimming rats.

METHODS

Animals and housing. Male Wistar rats weighing 320–350 g at the beginning of the experiments were used. The animals were individually housed in Plexiglass cages (25 × 25 × 30 cm) at room temperature (20 ± 2 °C), and had continuous access to food (Hope Farm chow) and water. The rats were maintained on a 12:12 h light–dark regime (07.00–19.00 h: lights on), and they were handled and weighed every day at 09.00 h.

Surgery. All animals were provided with two permanent heart catheters two weeks before the experiments. This and all other surgery was performed under ether anaesthesia. The heart catheters allowed blood sampling and infusions in freely moving, undisturbed rats. This method has been extensively described before (Steffens 1969). One week after insertion of the heart catheters, the rats were hepatically denervated or sham operated.

Denervation and adrenomedullation. A laparotomy was performed in the midline. Using micro-surgical instruments under an operating microscope (Leitz with 25 times magnification), the hepatic artery was

denervated close to the hilum, but deliberately sparing the hepatic vagal nerves (Holmin *et al.* 1984). A myelin specific dye (Toluidin Blue) was used to visualize the peri-arterial nerves. Sham operated animals were treated similarly, except for dividing the nerves. Bilateral adrenomedullation was performed by flank incision. The adrenal medullae were removed by surgical enucleation of the medulla.

Physical exercise. Exercise was performed in a pool made of stainless steel (length 3.00 m, width 0.40 m and depth 0.90 m) filled for 70% with water with a temperature of 33 ± 2 °C. The pool was equipped with a starting platform (33 × 37 cm) placed 2 cm above the water level. This starting platform could be lowered into the water down to the bottom of the swimming pool. A water pump (Loewe Silenta, FRG) provided a counter current of 0.22 m s⁻¹ that forced the animals to swim continuously. At the end of the exercise period, a removable resting platform (20 × 37 cm) at the upstream side of the swimming pool was offered to the swimming rat. To eliminate emotional stress of novelty, the rats were accustomed to swimming and blood sampling several times. The rats readily learned to climb on the lighted and warmed platform within 2 minutes after presentation.

Experimental set up. All experiments were performed during the light period between 10.00 and 13.00 h. On the experimental day, food was removed from the home cage 1.5 h before the start of the experiment. Forty-five minutes before the first blood sample, the cardiac catheters of the animals were connected to tubings for blood sampling and infusion (in mm: 300 length, 1.25 OD, and 0.75 ID). Throughout the experiments, 12 blood samples were taken for determination of the concentrations of glucose, FFA, catecholamines, insulin, and glucagon. To avoid diminution of the blood volume after blood sampling, a transfusion was given of a similar amount of citrated donor blood (10%). Donor blood was obtained from undisturbed rats with permanent heart catheters. Over a ten minute period, two blood samples (time points $t = -11$ min and $t = -1$ min, 0.5 ml and 0.4 ml, respectively) were taken in the home cage of the rat, to measure baseline levels of the blood components. The animals were then transferred to the starting platform of the swimming pool ($t = 0$ min). Blood samples were taken at $t = 1.5$ min (0.15 ml) and $t = 10$ min (0.6 ml). Immediately after withdrawal of the last blood sample, the platform was lowered to the bottom of the pool and the rats were forced to swim for 15 min. During swimming, blood samples were taken after 1, 5, 10 and 15 min, i.e. at $t = 11$ min (0.6 ml), 15 min (0.4 ml), 20 min (0.6 ml) and 25 min (0.5 ml). Immediately after the last blood sample, the resting platform was offered to the animal. On the resting platform, blood samples were taken at $t = 27$ min (0.6 ml), 32 min (0.5 ml), 37 min (0.4 ml) and 47 min (0.5 ml).

Experiments. Sham operated and liver denervated animals were subjected to four consecutive experiments. Ten days after hepatic denervation or sham operation, a control swimming experiment was performed to investigate the effect of hepatic denervation. This experiment was designated Experiment 1. Both groups of animals received an infusion of saline (0.1 ml min^{-1}) during exercise. In this and the following experiments, the infusion started as soon as the animals started to swim and was terminated after 15 minutes when the animals climbed on the resting platform.

Second, at day 14, Experiment 2 was performed in which an intravenous infusion of somatostatin (Sigma, $0.25 \mu\text{g ml}^{-1}$ in $0.1 \text{ ml saline min}^{-1}$) was given to both groups of animals. The dose of somatostatin was selected from previous experiments in rats (Ekelund *et al.* 1984). This experiment was performed to investigate a possible role of glucagon in the glycaemic response to exercise in hepatically denervated rats, since somatostatin is a powerful inhibitor of glucagon secretion (Gerich *et al.* 1975).

At day 15, both groups of animals were adrenalectomized to exclude adrenal A as a possible compensatory factor. Thereafter, Experiments 1 and 2 were repeated on day 25 and 29, and designated Experiments 3 and 4, respectively.

Four hours after Experiment 4, all animals were sacrificed with O_2/CO_2 . One blood sample was taken by heart puncture and biopsies were taken from the pancreas and from the central and superficial part of the liver. In these samples, concentration of NA was measured to quantify the efficiency of denervation.

Chemical determinations. Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing EDTA, $10 \mu\text{l}$ heparin (500 U ml^{-1}) and a trypsin inhibitor (Aprotinin NOVO, Novo Res. Inst., Bagsvaerd, Denmark) for preservation of glucagon. Blood glucose ($50 \mu\text{l}$ blood) was measured by the ferricyanide method of Hoffman (Technicon Auto Analyzer TMII). The remaining part was centrifuged for 15 min at 5000 rpm at 4°C . The supernatant was divided into three parts: $100 \mu\text{l}$ were immediately stored at -70°C for catecholamine measurements, $100 \mu\text{l}$ were used for the FFA assay and the remaining part was stored at -20°C for insulin and glucagon measurements. Tissue samples were quickly frozen in liquid nitrogen and stored at -70°C .

Determination of plasma catecholamine concentrations was performed by high pressure liquid chromatography (HPLC) in combination with electrochemical detection (ECD). This method has been described before (Scheurink *et al.* 1989a). For determination of tissue NA concentrations, 10–25 mg was cut from frozen tissue and was homogenized in $300 \mu\text{l}$ HClO_4 ($0.1 \text{ N} + 0.05\% \text{ EDTA}$) for 5 min (0°C). The homogenate was centrifuged for 10 min at

10000 r.p.m. and NA concentrations were determined by HPLC/ECD. Plasma FFA were extracted and photometrically determined (Antonios 1965). Plasma insulin and glucagon were determined by specific and sensitive radioimmunoassays (Novo Res. Inst., Bagsvaerd, Denmark) (Heding 1971, Scheurink *et al.* 1989b). As standards, rat insulin and porcine glucagon were used. The antiserum used for the determination of glucagon is specific for pancreatic glucagon.

Data analysis and statistics. Data are expressed as mean \pm SE. Wilcoxon matched-pairs signed rank test was used when levels of blood components were compared with the baseline value in the home cage ($t = -1 \text{ min}$). Two-way analysis of variance with repeated measures (ANOVA of SPSS\PC+) over the whole period was applied to determine significant differences between the liver denervated animals and the SHAM group. Further analysis was done by Mann-Whitney-U tests to determine the sources of significance obtained by ANOVA. A probability level of $P < 0.05$ was taken as statistical significance for all tests.

RESULTS

Experiment 1

The results of Experiment 1, in which both groups of animals were subjected to exercise, are presented in Figure 1. Baseline levels of the blood components were similar in the liver denervated (LDX) and sham-operated (SHAM) rats. Exercise increased blood or plasma levels of glucose, NA, A and glucagon, and decreased plasma insulin levels in both groups of animals. Plasma FFA rose immediately following the exercise. No statistical differences in the exercise-induced changes of blood components were observed between LDX and SHAM rats. The exercise-induced changes were similar to the alterations seen in previous studies (Scheurink *et al.* 1989a, 1989b, Scheurink & Steffens 1990).

Experiment 2

In Experiment 2, the two groups of animals were subjected to an intravenous infusion of somatostatin during exercise. The results are depicted in Figure 2. Baseline levels of the blood components were similar in the LDX and SHAM rats.

Infusion of somatostatin during exercise markedly influenced the alterations in blood glucose, plasma NA, A, insulin and glucagon in SHAM animals. Thus, somatostatin infusion signifi-

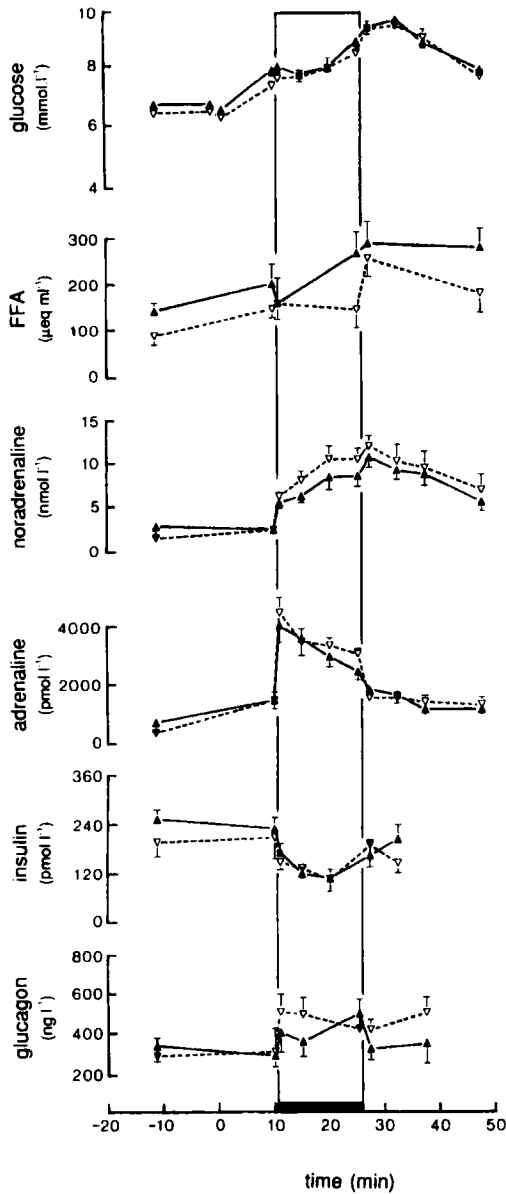


Fig. 1. Effect of sympathetic liver denervation (black triangles; $n = 7$) and sham operation (open triangles; $n = 6$) on concentrations of blood glucose, plasma FFA, NA, A, insulin and glucagon before, during and after swimming in the rat. During swimming (denoted by a black bar on the time axis), all animals received an infusion of saline (0.1 ml min^{-1}). All data are expressed as mean \pm SE.

cantly suppressed the exercise-induced increase in glucagon which was seen in Experiment 1 (significant at time points $t = 25$ and 27 min). Plasma insulin and blood glucose levels were also

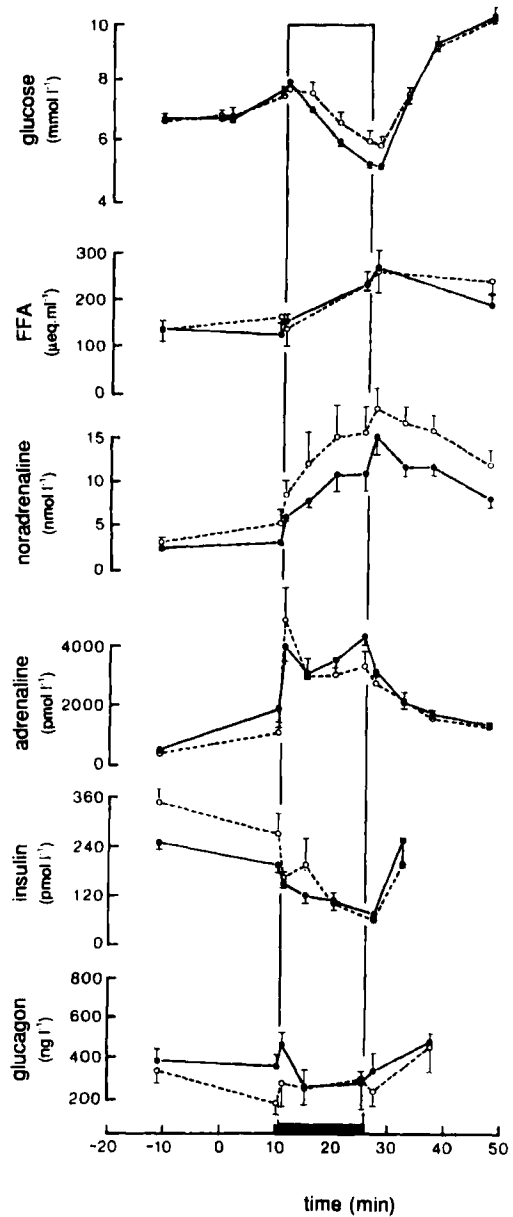


Fig. 2. Effect of somatostatin on concentrations of blood glucose, plasma FFA, NA, A, insulin and glucagon before, during and after swimming in liver denervated rats (black circles; $n = 7$) and sham-operated rats (open circles; $n = 6$). Somatostatin ($0.25 \mu\text{g min}^{-1}$) was administered (0.1 ml min^{-1}) during swimming (denoted by a black bar on the time axis). All data are expressed as mean \pm SE.

lower in the SHAM animals when compared with the saline infusion in Experiment 1 (for insulin, significant at time points $t = 17$, and for

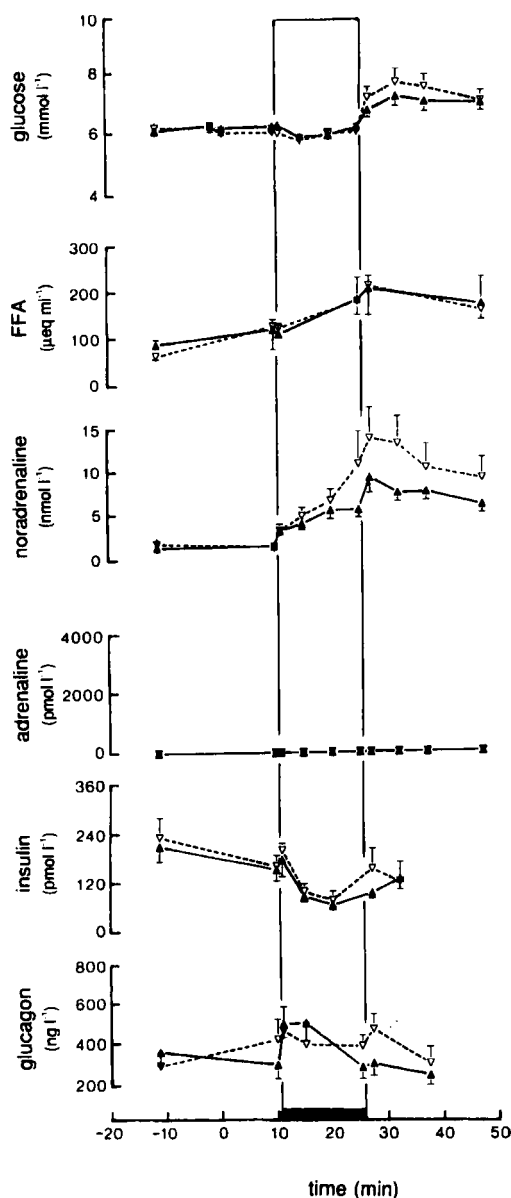


Fig. 3. Effect of adrenalectomy on concentrations of blood glucose, plasma FFA, NA, A, insulin and glucagon before, during and after swimming in liver denervated rats (black triangles; $n = 7$) and sham-operated rats (open triangles; $n = 6$). During swimming (denoted by a black bar on the time axis), all animals received an infusion (0.1 ml min^{-1}) of saline. All data are expressed as mean \pm SE.

glucose, significant at time points $t = 20, 25, 27$ and 32 min). The exercise-induced increases of plasma NA and A concentrations were enhanced as a result of somatostatin infusion (significantly

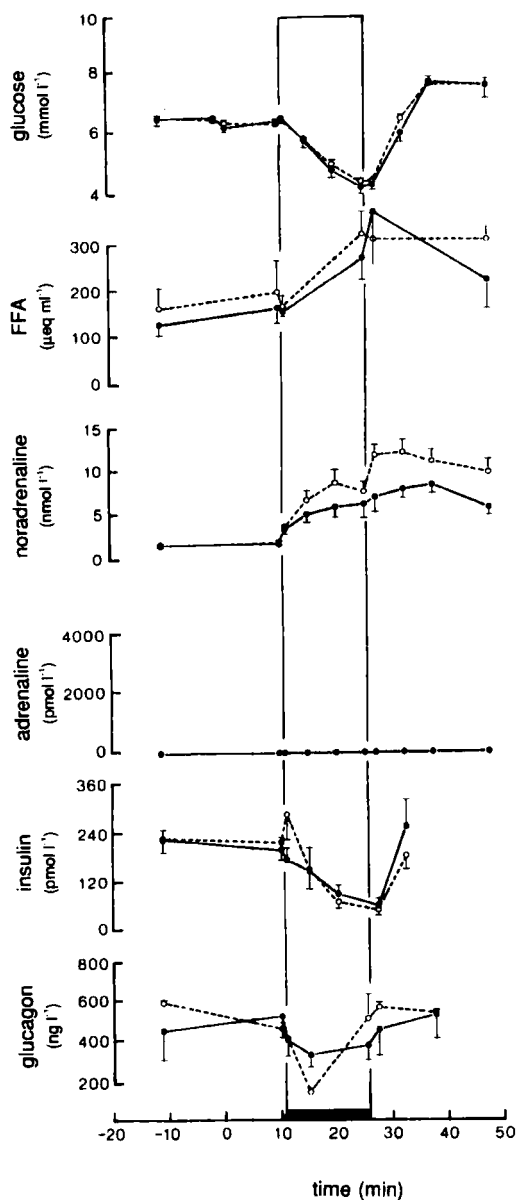


Fig. 4. Effect of somatostatin on concentrations of blood glucose, plasma FFA, NA, A, insulin, and glucagon before, during and after swimming in adrenalectomized liver denervated rats (black circles; $n = 7$) and adrenalectomized sham-operated rats (open circles; $n = 6$). Somatostatin (0.25 μg min^{-1}) was administered (0.1 ml min^{-1}) during swimming (denoted by a black bar on the time axis). All data are expressed as mean \pm SE.

higher than the data in Experiment 1 at time points $t = 27$ and 32 min for NA and time point $t = 27 \text{ min}$ for A).

Table 1. Noradrenaline concentrations in tissue and plasma in liver denervated and sham-operated rats

| | Liver | | Pancreas | Plasma |
|-----------------------------------|--------------------|-------------------|-----------------|---------------------|
| | Central | Superficial | | |
| Sham operated rats ($n = 6$) | 73.7 ± 15.1 | 42.3 ± 4.8 | 469 ± 55 | 129.5 ± 21.2 |
| Liver denervated rats ($n = 7$) | 44.4 ± 11.4 | 6.6 ± 2.1 | 490 ± 55 | 136.0 ± 21.3 |
| % Reduction | 40.0 | 84.4* | -4.5 | -5.0 |

Concentrations of tissue NA in liver and pancreas are given as ng g^{-1} tissue and the concentration of plasma NA is given as nmol l^{-1} . Samples were taken 4 h after Experiment 4. The reduction of NA due to liver denervation is denoted by % reduction. Values are mean \pm SE. * Denotes a significant change in the liver denervated group compared with the sham operated group.

In the LDX rats, somatostatin infusion and exercise led to blood glucose levels that were even lower than the markedly reduced levels observed in the somatostatin-infused SHAM animals (significant at time point $t = 27$ min). The LDX rats showed also an enhanced exercise-induced increase of plasma A concentrations as a result of somatostatin infusion (significantly higher than the data in Experiment 1 at time points $t = 25$, 27 and 37 min). However, the exaggerated increase of plasma NA, which was seen in the somatostatin-infused SHAM animals, could not be observed in the LDX rats (significant at time points $t = 11$ and $t = 22$ min). No differences between LDX and SHAM's were found in the concentrations of plasma FFA during somatostatin infusion.

Experiment 3

The results of Experiment 3, in which both groups of adrenalectomized animals were subjected to exercise, are presented in Figure 3. Plasma concentrations of A were below detection levels (lower than 25 pmol l^{-1}) after adrenalectomy. Baseline levels of the other blood components were similar in LDX and SHAM rats, and were not different from SHAM baseline levels in Experiment 1.

Adrenalectomy markedly reduced the exercise-induced increases of blood glucose and plasma NA in the SHAM rats (significant at time points $t = 10$, 11, 15, 20, 25, 27, 32 and 37 min for glucose, and $t = 15$ min for NA). Plasma FFA, insulin and glucagon in ADMX

SHAM rats during and after exercise were not different from the SHAM animals in Experiment 1. No differences were observed between the LDX and SHAM groups after adrenalectomy.

Experiment 4

The results of Experiment 4, in which both groups of adrenalectomized animals were subjected to an intravenous infusion of somatostatin during exercise, are presented in Figure 4. Plasma concentrations of A were below detection levels (lower than 25 pmol l^{-1}). Baseline levels of all other blood components were similar in LDX and SHAM rats, and were not different from SHAM baseline levels in Experiment 1.

As in Experiment 2, infusion of somatostatin during exercise markedly influenced the alterations in blood glucose, plasma NA, insulin and glucagon in both groups of animals. Somatostatin infusion significantly suppressed the exercise-induced increase in glucagon, as seen in Experiment 3 (significant at time point $t = 15$ min for the SHAM animals). Plasma insulin and blood glucose levels were also lower in the SHAM animals when compared with the ADMX saline infusion in Experiment 3 (for insulin, significant at time point $t = 27$ min, and for glucose, significant at time points $t = 20$, 25, 27 and 32 min). The reduction in the exercise-induced increase in glucose during somatostatin infusion was even larger when the data of the ADMX SHAM animals were compared with the SHAM animals in Experiment 2. In contrast to the exaggerated increase of NA observed in the

SHAM animals in Experiment 2, no significant differences were seen between SHAM animals in Experiments 3 and 4.

Finally, no significant differences in blood glucose, plasma FFA, insulin and glucagon during somatostatin infusion were found between the ADMX SHAM and the ADMX LDX rats, whereas plasma NA levels were lower in the ADMX LDX animals (significant at time point $t = 27$ min) in comparison with the ADMX SHAM rats.

Noradrenaline measurements in tissue

The concentrations of NA in liver, pancreas and plasma are shown in Table 1. In the LDX rats, denervation caused a reduction in NA content of respectively 40% and 84% ($P < 0.05$) in the central and superficial part of the liver when compared with the SHAM animals. No differences in concentrations of plasma NA and pancreatic NA were observed between LDX and SHAM animals.

DISCUSSION

The present study was designed to investigate the relative importance of hepatic sympathetic innervation and possible compensatory mechanisms in the glycaemic response to exercise. Concentrations of blood glucose, plasma FFA, A, NA, insulin and glucagon were monitored under control conditions and in hepatically denervated, adrenodemodulated and somatostatin infused swimming rats.

We found that administration of somatostatin abolished the glucagon response and reduced the blood glucose during exercise, indicating that glucagon is a powerful mediator of the glycaemic response during swimming. Also adrenodemodulation reduced the exercise-induced increase in blood glucose levels, indicating that A is another important mediator of glucose release during swimming. Finally, hepatic sympathetic denervation led to a more pronounced reduction in blood glucose in somatostatin- than in saline-infused animals in the presence, but not in the absence, of the adrenal medulla. Provided that the observed reduction in peripheral glucagon levels reflects also abolished portal glucagon response, our results suggest that intact liver nerves are contributing to the glycaemic response to exercise when glucagon is suppressed.

Infusion of somatostatin in SHAM animals led to a compensatory increase in sympatho-adrenal outflow reflected by enhanced plasma NA and A concentrations. This suggests that the effects of suppressed glucagon release are compensated for by an exaggerated outflow of A from the adrenal medulla and NA from the nerve terminals of the sympathetic nervous system (Issekutz & Vranic 1980, Khalil *et al.* 1986). Liver denervated rats did not respond with an enhanced outflow of NA to somatostatin infusion. As a consequence, blood glucose levels dropped below SHAM values in somatostatin-infused LDX rats. These data suggest that sympathetic nerves to the liver are an important source for the compensatory outflow of NA in somatostatin-infused exercising rats. Furthermore, these data suggest that, under special circumstances, such as when glucagon secretion is suppressed in the presence of the adrenal medulla, liver nerves do contribute to the glycaemic response to exercise.

Adrenodemodulation reduced the glycaemic response to exercise, which emphasizes the contribution of adrenal A to hepatic glucose production (Seydoux *et al.* 1979, Exton 1985, Sonne *et al.* 1985). However, blood glucose levels did not drop below baseline levels during exercise as seen during the somatostatin infusion. As a consequence, an effect of liver denervation and/or a compensatory increase in plasma NA could hardly be observed in adrenodemodulated exercising rats. A compensatory increase in plasma NA was neither observed when blood glucose levels were dramatically reduced in somatostatin-infused adrenodemodulated rats. It may be hypothesized that an increased outflow of adrenal A is required to allow a maximal outflow of NA from sympathetic nerve endings. This explanation is in agreement with earlier findings showing that adrenal A stimulates sympathetic outflow via a β_2 -adrenoceptor pre-synaptic mechanism (Majewski 1983, Remie *et al.* 1988, Scheurink *et al.* 1989b). Other explanations, for example that the increase in NA levels is the result of an enhanced outflow of NA from the adrenal medulla, seem unlikely since a number of studies have revealed that adrenal outflow of NA does not contribute to the alterations of NA in plasma (Richter *et al.* 1980, Bereiter *et al.* 1986).

In conclusion, the data of the present study demonstrate that pancreatic glucagon and

adrenal A serve as the predominant factors mediating the glycaemic response to exercise in rats. The role of sympathetic nerves innervating the liver in the glycaemic response to exercise is minor and becomes evident only when glucagon secretion is suppressed in the presence of the adrenal medulla. The requirement of suppressed glucagon secretion to observe an effect of the liver nerves in this respect, might explain the discrepancy between studies in liver denervated animals that failed to provide evidence for a role of hepatic sympathetic nerves in the mobilization of glucose from the liver (Mikines *et al.* 1985, Sonne 1989, Wasserman *et al.* 1990) and studies showing an increased hepatic glucose outflow after direct stimulation of the sympathetic nerves innervating the liver (Garceau *et al.* 1983, Shimazu 1983). Finally, it has to be mentioned that possible compensatory mechanisms, such as increased glucagon (Lindfeldt *et al.* 1987) and/or adrenoceptor sensitivity (Larsson *et al.* 1989), might have compensated for the loss of sympathetic innervation in the LDX animals, which would lead to an underestimation of the role of sympathetic liver nerves in the glycaemic response to exercise in Experiment 1. Increased adrenoceptor sensitivity seems, however, unlikely, since we recently demonstrated that the glycaemic responses to intravenous infusions of adrenaline and noradrenaline were similar in liver denervated and control rats (Lindfeldt *et al.* 1993).

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